Effectiveness of Combination of Tamarind and Saffron as Antidiabetic

Hilda[1], Pratiwi Rukmana Nasution[2]

[1,2] Politeknik Kesehatan Kementerian Kesehatan Medan, Jl. Jamin Ginting Km 13,5 Kel. Lau Cih, Medan Tuntungan

Email corespondensi : hildasherman.apt@gmail.com

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Abstract

According to the International Diabetes Federation, 537 million people are currently living with diabetes. There are 316 million people with impaired glucose tolerance at high risk, an alarming number expected to reach 783 million people by 2045. Society's tendency to return to nature is a prospective condition for developing research on biodiversity-based plants. Based on the research results, tamarind leaves are rich in flavonoids, saponins, and tannins, and saffron contains the active compounds crocin, crocetin, and precision, which can be candidates for antioxidants that have a significant effect on blood glucose levels. This antidiabetic research includes research live (test animals) by measuring blood glucose levels. The results showed that tamarind leaf extract and saffron were effective as antidiabetics in male mice induced with Streptozotocin. All extract groups tested single doses of EDAJ, ES, and combination doses of EDAJ and ES ((75:25), (50:50) (25:75)) showed the potential to reduce blood sugar levels >50%. The ES group with a single dose of 100:0 showed significant effectiveness in reducing blood sugar levels from the 6th day until the end of the study. These results are then expected to provide information to the public regarding the potency, effective dose, and working mechanism of using tamarind and saffron as antidiabetics so that they can be a solution to improve the quality of life of diabetes patients, control non-communicable diseases and reduce treatment costs.

Keywords: Crocus sativus, Diabetes mellitus, Mus musculus, Saffron, Tamarind leaf

INTRODUCTION

The most prevalent disease or metabolic problem in society is diabetes mellitus. Diabetes mellitus is a degenerative disease that develops due to the body's function, structure, or tissue gradually deteriorating with aging or environmental factors. Hyperglycemia is commonly found in people with diabetes. Diabetes mellitus can be treated by regulating diet and regular exercise, oral antidiabetic drugs such as sulfonylureas and biguanides, and insulin injections (DiPiro et al., 2008). However, the drugs circulating in the market, besides having a relatively expensive price and being used for a relatively long time, also have considerable side effects. Therefore, people are always trying to find alternative treatments, for example, by using traditional medicines, besides being easy to obtain, relatively low prices, and having smaller side effects compared to synthetic drugs (Abu-Iznei et al., 2020).

The tendency of society to *back to nature* and, according to government policy, "Saintifikasi Jamu" is a very prospective condition for developing research on Indonesian biodiversity-based plants (Yassir et al., 2014). Among the plants with the potential as an antidiabetic agent are tamarind leaves *(Tamarindus indica)* and the saffron plant (*Crocus*

sativus). These plants are rich in secondary metabolites such as flavonoids, saponins, and tannins which are thought to have the potential to lower blood glucose, prevent insulin resistance, and inhibit the enzyme alpha glucosidase. The ethanol extract of tamarind leaves has the effect of lowering blood glucose levels in alloxan-induced mice. The hypoglycemic effect increases with increasing doses in the range of 100 mg – 200 mg/200 kg BB (Chigurupati et al., 2020).

Tamarind leaf extract has been identified to contain chemical compounds such as flavonoids, tannins, glycosides and saponins. Tamarind water extract can inhibit weight gain, reduce cholesterol, triglyceride, and blood glucose levels, and reduce the risk of insulin resistance in animal models induced by high diets carbohydrates (Rimta Barus et al., 2021). Saffron is a spice that comes from dried flower buds. The spice is an original additive commonly used in Iran. Over the years, saffron has been the most expensive traditional Iranian spice due to planting, caring, harvesting, and processing, which is quite sufficiently long and hard. This plant contains three main secondary metabolites: crocin, picrocrocin, and safranal. The three metabolites produce the distinctive color, taste, and aroma of the saffron extract, which acts as a Strong antioxidant to inhibit glucose levels in the blood. Extract research results saffron ethanol at 20 mg/kgBB and 40 mg/kgBB can reduce streptozotocin-induced rat blood glucose levels after 4 weeks (Samarghandian et al., 2017).

Based on the description above, researchers are interested in further testing the effectiveness of Indonesian plant extracts, namely tamarind leaves (*tamarindus indica*L.) combined with saffron (*Crocus sativus)*, as an antidiabetic agent in male mice (*Mouse muscle*) induced by streptozotocin.Compared to single use, researchh on the combination of tamarind and saffron leaves aims to maximize the antidiabetic potential between each plant with different substance contene. Combining these two natural ingredients is expected to reduce fewer side effects than their single use (Anugrahini et al., 2021).

In this study, the extract's mechanism of action will also be observed through several test parameters, including the body weight of the test animals, blood glucose levels, and insulin resistance tests. So that this research can be the development of empowering Indonesian plants as scientific-based medicinal plants for diabetes mellitus, so that it is expected to provide information related to effective doses, improving the quality of life of patients and efficiency of treatment costs.

METHODS

This type of research is an experimental method to determine the effect of giving a

treatment or treatment to research subjects. The research design used is *one group pretest-posttest design*. Where in this study design, there were groups given treatment (treatment), and then the results were observed (the treatment was the independent variable, and the results were the dependent variable) (Sugiyono, 2018).

The population of this study was mice given tamarind and saffron plant extracts. The sample in this study was mice which were divided into several groups. The distribution of the test animal groups is as follows; Normal group, comparison group (glibenclamide), tamarind leaf extract group, combined extract group of tamarind and saffron (75 : 25), (50 : 50), (25 : 75), and saffron extract group. The research was conducted at the Phytochemical Laboratory and Pharmacology Laboratory, Department of Pharmacy, Poltekkes Kemenkes Medan, Jl. Airlangga No. 20 Medan. This research was carried out for ± 10 months, from January to October 2021.

Simplicia is made by collecting tamarind leaves and saffron, draining and drying them until dry. Then the dry samples were mashed using a blender, weighed, and stored in a well-closed container. The extract was prepared by maceration with 96% ethanol. The extracted Simplicia was then filtered and concentrated using a rotary evaporator. Test Simplicia was determined at the Medanese Herbarium, University of North Sumatra. Characterization of Simplicia and extracts included testing for water content, ash content, ethanol soluble extract content, water soluble extract content, and specific gravity. The characterization was continued with the extract's phytochemical screening, which included examining alkaloids, saponins, quinones, tannins, saponins, flavonoids, and steroids/triterpenoids.

In this study, mice were generally divided into 7 groups. Each group of test animals consisted of 4 mice. The mice used were male mice (*Mus musculus*) with healthy body weights 20-30 g and 2-3 months old. The distribution of test animal groups includes: Normal group without streptozotocin induction and only given carrier. Comparison group with Streptzotocin-induced and administered groups glibenclamide at a dose of 2.6 mg/kg BB. Tamarind leaf extract group (100 : 0) where the group that was induced by streptozotocin and was given tamarind extract at a dose of 100 mg/kgBB. Combination group of tamarind leaf extract and saffron extract (75 : 25) where induced by streptozotocin and given a combined preparation of tamarind leaf extract and saffron extract at doses of 75 mg/kgBB and 20 mg/kgBB. Combination group of tamarind leaf extract and saffron extract (50 : 50) where induced by streptozotocin and given combination preparations of tamarind leaf extract and saffron extract 100 mg/kgBB and 80 mg/kgBB. A combination group of tamarind leaf extract and saffron extract (25 : 75) where induced by streptozotocin and given combination of tamarind leaf extract and saffron extract 25 mg/kgBB & 60 mg/kgBBg. Saffron extract group (0 : 100) were

induced by streptozotocin and given 80 mg/kgBB of saffron extract.

The mice were fasted for 18 hours (drinking water was still given) and the fasting blood sugar level was measured. After that, they were injected with streptozotocin solution intraperitoneally at a dose of 90 mg/kg and given a glucose drink to keep the blood glucose levels high / hyperglycemia. On the 3rd day, the fasting blood glucose levels of the mice were measured after being induced. If they were positive for diabetes, they were immediately given an ethanol extract of a combination of tamarind leaves and saffron. The mice used were when their blood sugar levels were ≥ 200 mg/dl. The mice in this study were a type 2 diabetes model that continued with the combination extract treatment for 9 days.

This data was analyzed using the statistical software SPSS 25.0 for Windows Evaluation Version. Statistical Analysis The data obtained, namely blood glucose levels with several measurements, were analyzed statistically. The statistical analysis used in this study was first seen whether the data was normally distributed by using the Shapiro Wilk test. Normally distributed data has a p value > 0.05. If the data is normally distributed, then proceed with the Homogeneity of Variance test. If p> 0.05 then it is stated that all data have the same variance. One-way ANOVA parametric continued test (*one way* ANOVA). After that, it was followed by a Post Hoc Test to see if there were differences between each treatment group.

RESULTS

Simplicia and Extraction Results

As much as 1 kg of fresh tamarind and 300 grams of saffron are cleaned of dirt, dried and powdered. Then maceration was carried out using ethanol. Simplicia weight and maceration yield results can be seen in Table 1.

Table 1. Weight of Simplicia and Yield of Test Extract

Simplicia	Fresh (g)	Powder (g)	Extraction Yield (g)
Tamarind leaves	1500	540	133
Saffron	500	350	0,030

Phytochemical Screening

Phytochemical screening is carried out to determine the characteristics of the bioactive components of a crude extract that have toxic effects or other pharmacological effects that are useful when tested with biological systems or bioassays (Harborne, 1987). The results of the phytochemical screening can be seen in Table 2.

Table 2. Result of Simplicia Phytochemical Screening

Simplicia	Phytochemical screening test results				
	Flavonoid	Alkaloid	Tannin	Triterpenoids	Saponin
Tamarind leaves	+	-	+	+	+
Saffron	+	+	+	-	+

The results of the phytochemical screening above show that the tamarind leaf simplicia and saffron contain flavonoids, saponins, tannins, and triterpenoids. These metabolites' content is related to this plant's pharmacological properties. The presence of flavonoids and tannins in the tamarind leaf plant strongly supports this plant as an antidiabetic agent (Muhamadiyah et al., 2013). Based on previous research, saffron also contains a special substance, namely crocin, picrocrocin, and safranal which are high antioxidant substances to inhibit glucosidase enzymes in the small intestine (Cerdá-Bernad et al., 2020).

Antidiabetic Test Results

Procedure for testing the antidiabetic effects of ethanol extract from tamarind leaves and saffron on male mice *(Mus musculus)* Its implementation has been approved by the research ethics committee of the Medan Polytechnic of the Ministry of Health. The results of measuring the blood glucose levels of the test animals can be seen in Table 3 below.

Table 3. Test Animal Blood Glucose Levels

Group	Blood Glucose Levels (mg/dl)					% Change in Blood Glucose Levels			
	T0	Induction	T3	T6	T9	T0	T3	T6	T9
NORMAL	87,59	107,77	106,48	104,68	102,03	100,00	105,60	119,50	122,57
INDUCTION	86,50	326,85	258,44	231,53	213,36	100,00	280,32	269,75	249,00
COMPARISON 2.6 mg/KgBB	92,40	321,20	212,25	111,33	100,75	100,00	276,14	120,33	109,13
EDAJ (100%) 100 mg/KgBB	94,01	305,01	212,97	131,84	98,00	100,00	275,77	140,52	104,46
EDAJ+ES (75:25) 75 mg/kgBB & 20 mg/kgBB	92,19	311,20	199,21	128,79	93,97	100,00	254,12	140,72	102,42
EDAJ+ES (50:50) 100 mg/kgBB & 80mg/kgBB	91,79	305,95	187,29	105,24	80,50	100,00	240,36	114,79	87,76

EDAJ+ES (25:75) 25 mg/kgBB & 60 mg/kgBB	92,39	326,39	172,66	103,00	78,00	100,00	229,60	111,62	84,54
ES (100%) 80 mg/KgBB	93,35	308,82	140,75	98,25	67,50	100,00	226,24	105,87	72,83

Information:
EDAJ : Tamarind leaves Ethanol Extract
ES : Saffron Ethanol Extract

The blood glucose levels measurements of the test animals were carried out at T0, T3, T6, and T9 on the study day after previously the animals fasted for 8-12 hours while still being given sufficient water. T0 is the time of measuring the blood glucose levels of the test animals before being given induction. After measuring the animals' blood glucose levels under normal conditions (T0), all groups of test animals were given streptozotocin (ip) and 10% glucose solution, except the normal group. After that, the test animals were given standard food and drink and still given 10% glucose solution. On the 2nd day of the study, the blood glucose levels of the test animals were checked again (Induction T).

Test animals that showed an increase in blood glucose levels above normal (≥124 mg/dl) were used as test animals in this study. According to Table 4.3, it can be seen that all the test animals used had blood glucose levels ≤ 124 mg/dl. After being given an induction, all groups of test animals showed increased blood glucose levels beyond normal limits. The test method used was a curative principle, in which the test animals were induced with streptozotocin (ip) at a dose of 90 mg/kgBB and 10% glucose solution (po) to create hyperglycemia conditions. Only test animals that showed an increase in blood glucose levels >140 mg/dl after being induced were used as test animals in this study. Streptozotocin induces a substance that can cause alkylation, which directly methylates DNA causing DNA strand breaks and damage to pancreatic Langerhans beta cells. STZ induction for a maximum of 2 x 24 hours will make mice with type 2 DM models more than that will make them type 1 DM.

The normal group that was only given the carrier, namely Na-CMC, did not show changes in blood glucose levels that exceeded the normal blood glucose levels limits compared to the other groups. This shows that the carrier has no effect as an inductor and reduces blood glucose levels in test animals. The positive control was glibenclamide at a 2.6 mg/KgBW dose. Glibenclamide is a diabetes drug in the sulfonylurea class which can increase insulin secretion. The mechanism of action is to block K+ channels in pancreatic beta cells, causing depolarization and opening of Ca2+ channels, the entry of Ca2+ ions into pancreatic beta cells causes an increase in insulin secretion. Long-term use can cause hypoglycemia

(Gumantara et al., 2017).

Test animals that were declared hyperglycemia were then given an intervention by administering carrier preparations (CMC Na suspension) and test extract preparations (ethanol extract of tamarind leaves, and saffron). The test extract preparations were administered orally for 6 days after the test animals were declared hyperglycemia. Blood glucose levels measurements were carried out every 3 days after the intervention of administering the test preparation using a glucose test strip. The results of the percentage change in the blood glucose levels of the test animals can be seen in Table 3. and Figure 1.

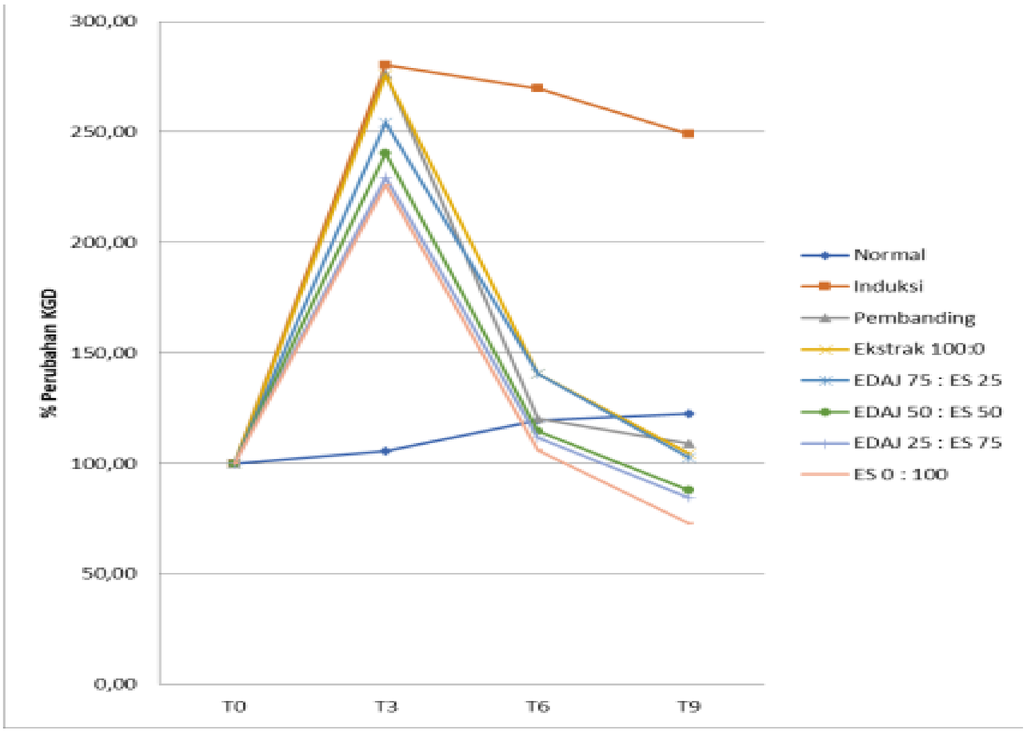


Figure 1. Percentage of changes in blood sugar levels of test animals at T0 and after being induced

Based on Figure 1. It can be seen that all groups of mice experienced changes in the decrease in blood glucose levels after being induced and given ethanol extract of tamarind leaves and saffron on the T3 to T9 measurement days, where the test group showed antidiabetic potential compared to the normal and the normal groups. Induction group significantly ($p<0.05$).

On the 3rd day of administration of the test extract, all treatment groups, single and combined, differed significantly from the induction group. This shows an improvement in blood glucose levels but has not yet reached a normal state. On the 6th day of the study, the EDAJ & ES 50:50 group was dosed at 100 mg KgBB and 80 mg/KgBB, the EDAJ & ES 20:75 group was dosed at 25 mg/KgBB and 60mg/KgBB, the ES 100 group, the dose was 80 mg/kgBB, showed the potential for reducing blood glucose levels which were significant compared to the Induction

group ($p<0.05$).

The comparison group showed the same potency in reducing blood glucose levels statistically as the 100mg/kg BW EDAJ group on the 6th day of the study. On the 9th day of the study, the EDAJ & ES 50:50 dose of 100mg/KgBB and 85 mg/KgBB, the EDAJ & ES 20:75 group, the dose of 25mg/KgBB and 60mg/KgBB, the ES 100 group, the dose of 80mg/kgBB, showed a significant difference ($p<0.05$) compared to the Induction group. The EDAJ 100:0 group at a dose of 100 mg/kg, the EDAJ 75: ES 25 group at 75 mg/kg and 20 mg/kg, and the comparison group showed almost the same potential to reduce blood glucose levels, reaching 71%.

All treatment groups were significantly different ($p<0.05$) from the normal control. However, there was a gradual decrease in blood glucose levels during measurement from the 3rd day and starting from the 6th day of the study, all test groups showed the potential to reduce blood glucose levels and at the end of the study all groups of test extracts showed the potential to decrease the blood glucose levels of test animals by >50%.

DISCUSSION

Diabetes mellitus is a disease characterized by hyperglycemia (increased blood sugar levels) which continuously varies, especially after eating. lesions on the basement membrane in electron microscopy examination (Maulana, 2008). Diabetes mellitus is a metabolic disorder caused by a lack of the hormone insulin. The insulin hormone is produced by a group of beta cells in the pancreas gland and plays a very important role in glucose metabolism in body cells. High glucose levels in the body cannot be absorbed and are not metabolized in cells. As a result, a person will lack energy and excess glucose levels will be excreted through the kidneys and excreted with urine. High blood sugar levels can damage blood vessels and nerves, and often cause complications such as heart disease, stroke, blindness and kidney disease (Shulman, 2000).

Tamarind leaves have a protective effect on pancreatic β seen from the histopathological results of rats. The induction used to increase glucose levels is alloxan, where the properties of alloxan are suitable for not damaging the pancreas permanently (Haryoto & Nur'aini, 2018). The use of tamarind is related to the activity of inhibiting α amylase activity by 90% (Funke et al., 2006), rich in amino acids Kuru (2014) and can reduce glucose levels, judging from the pancreatic histopathological test of the ethanol-water fraction has a clearer picture (Nurhayati et al., 2019). Giving a combination of ethanol extracts of tamarind and

soursop leaves can reduce blood glucose levels in alloxan-induced rats at a ratio of 25:75 by looking at the average hypoglycemic power (Safarini et al., 2019).

In an in vitro study, the ethanol extract of tamarind leaves showed 49% inhibition of the pancreatic lipase enzyme (Nasution et al., 2013). Other studies have also shown that tamarind flesh extract has a hypocholesterolemic effect. Research conducted by Lahamado et al., (2017) shows that tamarind leaf extract exhibits α-amylase inhibition, possibly being used as an alternative treatment for type 2 diabetes mellitus. Tamarind leaf extract has been identified to contain compounds of the chemical class of flavonoids, tannins, glycosides and saponins. Tamarind water extract can inhibit weight gain, reduce cholesterol, triglyceride, blood glucose levels, and reduce the risk of insulin resistance in animal models induced by a high-carbohydrate diet The water extract of tamarind pulp also showed a decrease in body weight, plasma cholesterol total, LDL, triglycerides, and HDL increases, decreased plasma leptin and fatty acid synthesis activity in high-fat diet-induced animal models. Flavonoid and polyphenolic compounds are thought to be responsible for this effect (Lahamado et al., 2017). The ethanol extract of fruit flesh also showed activity in lowering blood glucose levels, low density lipoprotein (LDL), triglycerides, and increasing high density lipoprotein (HDL) in alloxan-induced animal models (Tandi et al., 2019).

Saffron is a spice that comes from dried flower buds. The spice is an original additive commonly used in Iran and started bred in several countries. Based on the research and chemical analysis results which, the saffron plant does, it does demonstrate the presence of more than 34 volatile components, including terpenes, terpene alcohols, and their esters in saffron. The methodologies and techniques used to analyze saffron metabolites are chromatographic and spectroscopic techniques such as TLC, HPLC, GC-MS, LC-MS and NMR5 (Abu-Iznei et al., 2020). The four main ingredients present in Saffron are crocin (monoglycosyl or di-glycosyl polyene ester), crocetin (precursor of the natural carotenoid dicarboxylic acid of crocin), picrocrocin (precursor of monoterpene glycosides of safranal and product of zeaxanthin degradation) and safranal. Crocin, as a color giver to saffron is a carotenoid that dissolves in water because it has a high glycosyl content. Picocrocin is the main substance responsible for the taste of saffron and safranal is a volatile oil responsible for saffron's aroma (Rameshrad et al., 2017). The results of the ethanol extract of saffron with a dose of 20 mg/kgBW and a dose of 40 mg/kgBW were able to reduce blood glucose levels in rats streptozotocin induced after 4 weeks (Samarghandian et al., 2017).

The pancreas, which is called the stomach salivary gland, is an insulin-producing gland located behind the stomach. Inside are clusters of cells shaped like islands on the map, because they are called Langerhans islands which contain beta cells that secrete the hormone insulin which plays a very important role in regulating blood glucose levels. The insulin released by the beta cells can be likened to a key that can open the entrance of glucose into the cells, and then in the cells the glucose is metabolized into energy. If insulin is not available, then glucose in the blood cannot enter the cells because glucose levels in the blood cannot enter the cells with the result that glucose levels in the blood increase. This situation occurs in type 1 diabetes mellitus (Shulman, 2000).

In type 2 diabetes mellitus, the amount of insulin can be even more normal, but the number of insulin receptors (catchers) on the cell surface is less. The insulin receptor can be likened to a keyhole at the entrance to the cell. In type 2 DM, the number of keyholes is lacking, so even though there are many keyholes (insulin), because the keyholes (receptors) are lacking, less glucose enters the cell, so the cell lacks fuel (glucose), and glucose levels are low. Blood increases. Thus this situation is the same as the state of type 1 DM, the difference is that in type 2 DM, besides high glucose levels, insulin levels are also high or normal. In type 2 DM, a sufficient amount or more of insulin can also be found, but the quality is not good, so it fails to bring glucose into the cells. In addition to the causes above, DM can also occur due to impaired glucose transport in cells, so it fails to be used as fuel for energy metabolism (Shulman, 2000).

The most common cause of diabetes is type 2 diabetes mellitus, characterized by impaired insulin secretion or insulin action (insulin resistance) in target organs, especially the liver and muscles. Initially, insulin resistance has not caused clinical diabetes. At that time, the pancreatic beta cells could still compensate for this situation, hyperinsulinemia occurred, and blood glucose was still normal or slightly increased. Then after pancreatic beta cell incompetence occurs, clinical diabetes occurs, characterized by increased blood glucose levels that meet the criteria for the diagnosis of diabetes mellitus. Muscles are the most glucose users, so insulin resistance results in the failure of glucose uptake by muscles (DiPiro et al., 2008).

CONCLUSIONS

The combination of tamarind leaves and saffron has effectiveness as an antidiabetic in male mice induced by streptozotocin. All EDAJ test extracts: ES in all dose groups (100.0 (100 mg/kgBB), 75:25 (75 mg/kgBB & 20 mg/kgBB), 50:50 (100 mg/kgBB & 85 mg/kgBB), 25 :70 (25 mg/kgBB & 60mg/kgBB), 0:100 (85mg/kgBB)) shows the potential to reduce blood

glucose >50%. The ES 100:0 group (dose 85 mg/kgBW) significantly reduced blood glucose levels from the 6th day until the end of the study. Hopefully, this research can be the development of empowering Indonesian plants as scientific-based medicinal plants for diabetes mellitus, so that it is expected to provide information related to effective doses, improving the quality of life of patients and efficiency of treatment costs.

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